RNA Phase Transitions in Repeat Expansion Disorders

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This file contains Captions for Supplementary Tables 1-2 and Captions for Supplementary Videos 1-9.

Supplementary Table 1. Sequences of plasmids.

Supplementary Table 2. Sequences of DNA oligonucleotides and transcription templates.

Supplementary Video 1. Fluorescence recovery after photobleaching (FRAP) for RNA clusters *in vitro*. A section of 47xCAG RNA cluster, ~1 μm diameter, was photobleached and the fluorescence was monitored over time. The photobleached region did not exhibit appreciable recovery over ~10 min. Scale bar is 5 μm.

Supplementary Video 2. Base pairing interactions affect fluorescence recovery rates for phase separated DNA. T-90 (left), sequence S1 (center) or AT-45 (right) phase separate in the presence of spermine. ~1 μm diameter region was photobleached and the fluorescence recovery was monitored over time. The fluorescence recovery rate decreases with increasing base pairing. Scale bars represent 5 μm.

Supplementary Video 3. Real-time visualization of RNA foci formation. U-2OS cells were transduced with a 120xCAG RNA, tagged with 12xMS2 hairpins. RNA is visualized by co-expression of MS2CP-YFP. Expression of 120xCAG RNA was induced at t = 0 min and cells were visualized using confocal microscopy. Scale bar is 5 μm.

Supplementary Video 4. RNA foci are liquid-like and undergo fusion events. Cells were transduced to express MS2-tagged 47xCAG RNA, and RNA was visualized by co-expression of MS2CP-YFP. Two or more RNA foci in proximity coalesce in to a single punctum. A typical fusion event is marked by an arrow. Scale bar is 5 μm.

Supplementary Video 5. FRAP for 47xCAG RNA foci. 47xCAG RNA punctum was photobleached at t = 0 and the fluorescence recovery was monitored over time. Arrow indicates the site of photobleaching. Scale bar is 5 μm.

Supplementary Video 6. Partial bleaching for 47xCAG RNA foci. A region ~1 μm in diameter was photobleached in a 47xCAG RNA punctum and the fluorescence recovery was monitored over time. Arrow indicates the site of photobleaching. Scale bar is 5 μm.

Supplementary Video 7. FRAP for 47xCAG RNA foci after ATP depletion. Cellular ATP was depleted in cells expressing 47xCAG RNA. An RNA punctum was photobleached at t = 0 and the fluorescence recovery was monitored over time. Arrow indicates the site of photobleaching. Scale bar is 5 μm.

Supplementary Video 8. Effect of ammonium acetate on RNA foci. U-2OS cells expressing 47xCAG RNA were treated with 100 mM ammonium acetate at t = 0. RNA is visualized by co-expression of MS2CP-YFP. RNA foci disassemble within minutes after addition of ammonium acetate. Scale bar is 5 μm.

Supplementary Video 9. FRAP for 29xGGGGCC RNA foci. 29xGGGGCC RNA punctum was photobleached at t = 0 and the fluorescence recovery was monitored over time. Arrow indicates the site of photobleaching. Scale bar is 5 μm.